ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1997, p. 1802–1804 Vol. 41, No. 8
0066-4804/97/$04.00+0

Circadian Variation in Urinary Excretion of Ciprofloxacin after a Single-Dose Oral Administration at 1000 and 2200 Hours in Human Subjects

V. V. SARVESHWER RAO, D. RAMBHAU,* B. RAMESH RAO, AND P. SRINIVASU

University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506 009, India

Received 17 September 1996/Returned for modification 12 February 1997/Accepted 2 June 1997

Ciprofloxacin is routinely prescribed to treat a variety of infections, including those of the urinary tract. To achieve optimum therapeutic benefits of the drug, all of the factors which influence its pharmacokinetics and effectiveness need to be determined. This study investigated the urinary excretion kinetics of ciprofloxacin upon oral administration of a single dose of 250 mg at 1000 or 2200 h in 12 healthy human subjects in a crossover design. The urine samples were analyzed for unchanged ciprofloxacin by a sensitive high-performance liquid chromatography method. A significant decrease in the rate and extent of ciprofloxacin excretion following 2200 h (109.59 versus 53.8 mg [$P < 0.05$]) administration was observed. This result may be due to circadian changes in the factors affecting renal excretion and also probably metabolism of ciprofloxacin.

The chronopharmacokinetics of several drugs have been reported elsewhere (19). However, such studies of chemotherapeutic agents are scanty. Ciprofloxacin is a potent fluoroquinolone with a broad spectrum of activity. Its minimum effective concentrations are attainable in serum, body tissues, and urine following oral administration of the drug. It is used in a wide variety urinary- respiratory-, and gastrointestinal-tract infections. Because of its wide spectrum of activity, high degree of renal clearance (about 66%), and ability to become concentrated in urine (3), it is a drug of choice in the treatment of urinary-tract infections.

Since circadian rhythms in urinary pH, urine volume, glomerular filtration rate (13), and blood flow to the renal system (4) are reported, it is likely that these may alter the urinary excretion kinetics of drugs. We report the time-dependent renal excretion of ciprofloxacin in human subjects.

MATERIALS AND METHODS

This study was conducted with 12 healthy male volunteers (weight, 48 to 62 kg; height, 169 to 172 cm; and age, 19 to 32 years). Before selection, the health of the volunteers was checked by a thorough medical examination and standard laboratory tests. They were all nonsmokers and were instructed not to take alcoholic beverages or any other medication for 2 weeks before or during the study. The participants were confined to the laboratory on the study days. All of the volunteers were briefed about the study and gave written consent. The study was approved by the Institutional Ethical Committee. The subjects were randomly divided into two groups, and the study was conducted in a crossover design, with a washout period of 10 days between the treatments. Ciprofloxacin (250 mg as an immediate release capsule) was administered either at 1000 or 2200 h with a glass of water after approximately 10 h of fasting. No food or drink was permitted following 3 h, and the subjects remained in a sitting position. Regular meals were permitted after the stipulated time.

TABLE 1. Measured ciprofloxacin concentrations

Concn of spiked sample (μg/ml)	*n*	Measured mean concn (μg/ml)[a]	
		Within day	Between days
5	6	4.9 (4.2)	4.86 (4.8)
40	6	39.6 (3.8)	40.3 (4.5)

[a] Values in parentheses are coefficients of variation.

* Corresponding author.

Urine samples were collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 8.0, 11.0, and 15.0 h, and each time the total volume of urine excreted was measured. The urine samples were frozen until analysis was performed.

Assays. Urine samples were analyzed for unchanged ciprofloxacin by the modified high-performance liquid chromatography (HPLC) method described by Jehl et al. (12) on a Shimadzu HPLC unit with an octadecyl silane reverse-phase column and a UV spectrophotometric detector. The chromatographic conditions were as follows: mobile phase, acetonitrile-water (15:85) with 0.1% triethylamine (pH adjusted to 2.5 with 1 M orthophosphoric acid); flow rate, 1.5 ml/min; column temperature, −50°C; wavelength, 254 nm; and detector sensitivity, 0.005 AUFS (absorbance value corresponding to the full-scale recorder). Urine samples (0.5 ml) were transferred to a screw-cap test tube containing 0.2 ml of internal standard (Paracetamol) solution (10 μg/ml) in water, and the tube was mixed well. To this, dichloromethane (2 ml) was added and vortexed for 5 min, followed by centrifugation at high speed for 7 min. The upper aqueous phase was discarded, and 1 ml of phosphoric acid (pH 2.0) was added. Then, it was vortexed for 3 min followed by centrifugation at high speed for 7 min. The lower organic phase was rejected. A 25-μl volume of upper aqueous phase was injected into the column. A standard graph was prepared by adding 0.5 to 40 μg

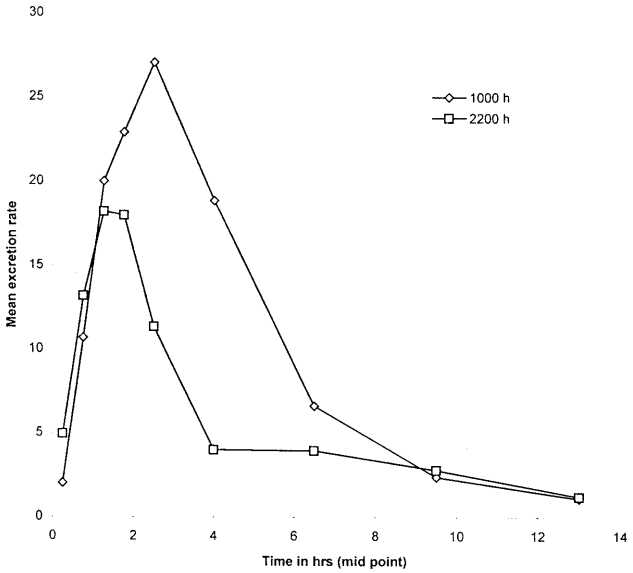

FIG. 1. Plots of the mean urinary excretion rate of ciprofloxacin versus time (midpoints) following oral administration of 250 mg of the drug at 1000 and 2200 h.

TABLE 2. Mean (± standard deviation) pharmacokinetic parameters obtained from urinary ciprofloxacin levels

Time of administration (h)	ER_{max} (mg/h)	$t_{1/2}$ (h)
1000	34.53 ± 15.62	2.67 ± 1.10
2200	17.64 ± 15.96	3.78 ± 1.76
P	<0.02	<0.05

of ciprofloxacin to 0.5-ml aliquots of urine from untreated volunteers. These standard samples were treated in the same manner as the test samples. The peak height ratios obtained at different concentrations of the drug were plotted against the drug concentrations. The slope of this plot, which was determined by least-squares regression analysis, was used to calculate the concentrations of ciprofloxacin in the unknown samples. The reproducibility of the assay method was tested by analyzing urine samples spiked with standard solutions of ciprofloxacin on different days as well as on a single day. The measured mean concentrations were as shown in Table 1.

Pharmacokinetic analysis. The excretion rate (ER) was calculated by dividing the amount of drug excreted between two sampling points by the time interval. Peak ER (ER_{max}) and the time to reach ER_{max} were obtained from a plot of ER versus time (midpoint) (10). The other pharmacokinetic parameters obtained were the elimination rate constant (k_{el}), half-life ($t_{1/2}$), and total amount of drug excreted. k_{el} was calculated from the slope of the terminal elimination phase of a semilogarithmic plot of ER versus time after subjecting it to linear regression analysis. Assuming the elimination to be a first-order process, $t_{1/2} = 0.693/k_{el}$.

The differences in the mean values of pharmacokinetic parameters were validated by a two-tailed paired *t* test at a probability level of 95%.

RESULTS

The plots of the mean urinary ER of ciprofloxacin versus time (midpoints) following oral administration of 250 mg of the drug at 1000 and 2200 h are shown in Fig. 1. The mean pharmacokinetic parameters obtained from urinary ciprofloxacin levels in all volunteers are given in Table 2. The mean ER_{max}s were 34.53 ± 15.62 and 17.64 ± 15.96 (± standard deviations) mg/h following administration at 1000 and 2200 h, respectively. The mean ER_{max} was significantly less ($P < 0.02$) when the drug was administered in the dark phase. After 3 h of drug ingestion, the mean cumulative amount of ciprofloxacin excreted was significantly lower for the 2200-h administration. In addition, the elimination $t_{1/2}$ for the 1000-h administration was lower and was significantly different from that of the 2200-h treatment ($P < 0.05$).

DISCUSSION

The antibiotics erythromycin (6) and rifampin (1) showed circadian rhythmicity in their pharmacokinetics, based on serum levels. Sulfasymazine, sulfanilamide, and sulfamethoxazole revealed circadian rhythms in their urinary excretion kinetics. As per the reports of Dettli and Spring (5), the sulfasymazine excretion rate in human subjects was more rapid during the day than the night, whereas sulfanilamide showed a similar trend that was, however, not statistically significant. In agreement with the present report, Rao and Rambhau (17) found in humans that the mean cumulative amount of sulfamethoxazole excreted in urine after 0600-h dosing was greater than those for the 1200-, 1800-, and 2400-h dosing times. Diurnal variations in the excretion of drugs other than antiinfectives were reported for several drugs. Studies of the chronopharmacokinetics of furosemide in rats revealed a significantly greater urine volume and excretion of sodium when the drug was administered at 1000 h than those when it was administered at 2200 h (8). Ollagnier et al. (16) reported that the amount of ketoprofen eliminated in the urine was significantly greater after administration at 0100 h than that at 0700 or at 1900 h.

Reinberg et al. (18) studied the circadian variations in the excretion of salicylates. For adults with diurnal activity (0700 to 2300 h), they observed that the excretion of salicylates was more prolonged when the drug was taken in the morning, while the opposite occurred in the evening. According to Mattok and McGilvery (15), the rate of urinary excretion of paracetamol was greater when it was taken at 0800 h than when it was taken in the evening. Urinary excretion of 5-(*p*-hydroxyphenyl)-5-phenylhydantoin was found to be larger during the day than during the night (9). Markiewicz and Semnowicz (14) observed the highest level of urinary excretion of D-xylose after 2 h absorption at noon. Urinary concentrations of cisplatin were greater, with its highest peak and largest area under the concentration-time curve occurring when the drug was administered in the morning (0600 h) compared with the evening (11).

Our observations regarding ciprofloxacin revealed a higher rate and extent of elimination of ciprofloxacin in urine upon its administration at 1000 h compared with those at 2200 h. We believe that this may be due to the diurnal variations in a variety of factors influencing the renal excretion of drugs. According to Koopman et al. (13) glomerular filtration is at its highest level during diurnal activity (112 ± 22 ml/min) and at its lowest level (86 ± 12 ml/min) during nocturnal sleep. In addition, a significant increase in urine flow and a considerable increase in tubular reabsorption of water during the night have been reported by these researchers.

The chronokinetics in the excretion of acidic drugs such as sulfasymazine, sulfamethoxazole, and salicylates are explained on the basis of circadian rhythms in the urinary pH, which is lower during the night (4, 20). The weakly acidic nature of ciprofloxacin might have contributed to the diurnal variation in its excretion.

Ciprofloxacin has been reported to undergo a first-pass ef-

TABLE 3. Means ± standard deviations of urine volumes (U_v), urinary concentrations (U_c), and cumulative amounts excreted (U_a) for ciprofloxacin following oral administration of 250 mg of the drug at 1000 and 2200 h.

Time after ingestion (h)	Value at 1000 h			Value at 2200 h		
	U_v (ml)	U_c (μg/ml)	U_a (mg)	U_v (ml)	U_c (μg/ml)	U_a (mg)
0.5	54 ± 70	20.8 ± 18	1 ± 0.65	42 ± 47	95 ± 75	2.47 ± 2.35
1	61 ± 88	377 ± 482	6.36 ± 7.17	39 ± 42	264 ± 171	8.63 ± 9.87
1.5	78 ± 92	845 ± 680	18.47 ± 16.45	67 ± 87	276 ± 203	17.73 ± 20.36
2	77 ± 94	462 ± 563	28.39 ± 21.02	78 ± 88	838 ± 214	22.56 ± 15.9
3	125 ± 87	334 ± 314	54.55 ± 17.42	124 ± 108	183 ± 174	25.27 ± 30.13
5	146 ± 133	237 ± 144	78.72 ± 29.79	218 ± 104	119 ± 134	32.31 ± 22.51
8	195 ± 275	172 ± 121	98.27 ± 39.42	218 ± 157	56 ± 52	43.15 ± 32.83
11	146 ± 203	94 ± 121	105.75 ± 41.5	263 ± 186	38 ± 36	50.34 ± 40.66
15	256 ± 457	152 ± 192	109.59 ± 44.99	210 ± 167	29 ± 28	53.78 ± 42.01

fect in the liver. Sulfo-ciprofloxacin appears to be the preferentially formed first-pass metabolite (7). The conversion of ciprofloxacin to sulfociprofloxacin involves sulfate conjugation, a reaction in which the xenobiotic combines with sulfate anion to produce sulfate ester (2, 21). Such conjugations are catalyzed by the enzyme systems closely associated with the sulfotransferases. Diurnal variations in the sulfotransferases in rat liver (nocturnally active), with a peak activity at 0900 h, have been reported elsewhere (22). It is possible that the levels of activity of the enzymes responsible for sulfate conjugation in humans are higher during the night, causing a rapid first-pass metabolism of ciprofloxacin. Thus, this may be one of the contributory factors to the decrease in the level of excretion of unchanged ciprofloxacin due to nocturnal administration.

In conclusion, although this study reports significant time-dependent changes in the urinary excretion of ciprofloxacin, such changes are not clinically relevant, since the urinary levels of the drug at all time points during the study are much higher than the MICs for the organisms causing urinary-tract infections (Table 3).

ACKNOWLEDGMENTS

We are grateful to the University Grants Commission, New Delhi, India, for financial assistance and M/S Cipla Limited, Bombay, India, for providing pure ciprofloxacin samples.

We wholeheartedly thank the study volunteers for their spirited participation.

REFERENCES

1. **Avachat, M. K., D. Rambhau, B. R. Rao, V. V. S. Rao, and J. V. Rao.** 1992. Chronopharmacokinetics of rifampicin. Indian J. Physiol. Pharmacol. **36:** 251–254.
2. **Belanger, P. M., M. Lalande, G. Labrecque, and F. Dore.** 1985. Diurnal variations in the transferases and hydroxylases involved in glucuronide and sulphate conjugation of rat liver. Drug Metab. Dispos. **13:**386–389.
3. **Bryan, K. V., D. R. P. Guay, and J. C. Rotschafer.** 1990. Clinical pharmacokinetics of ciprofloxacin. Clin. Pharmacokinet. **19:**434–461.
4. **Cal, J. C., C. Dorian, and J. Camber.** 1985. Circadian and circannual changes in nephrotoxic effects of heavy metals and antibiotics. Annu. Rev. Chronopharmacol. **2:**143–176.
5. **Dettli, L., and P. Spring.** 1966. Diurnal variations in the elimination rate of a sulphonamide in man. Helv. Med. Acta **33:**291–306.
6. **Disanto, A., D. Chodes, and F. Halberg.** 1975. Chronobioavailability of erythromycin test preparations assessed by each of four indices, time to peak, nadir and area. Chronobiologia **2**(Suppl. 1)**:**17.
7. **Drusano, G. L.** 1987. An overview of pharmacology of intravenously administered ciprofloxacin. Am. J. Med. **83**(Suppl. 4A)**:**339–345.
8. **Fujimura, A., and A. Ebihara.** 1986. Chronopharmacological study of frusemide in rats. Life Sci. **38:**1215–1320.
9. **Garrettson, L. K., and W. J. Jusko.** 1975. Diphenylhydantoin elimination kinetics in overdosed children. Clin. Pharmacol. Ther. **17:**481–491.
10. **Gibaldi, M., and D. Perrier.** 1982. Pharmacokinetics, 2nd ed. Marcel Dekker, Inc., New York, N.Y.
11. **Hrushesky, W., F. Levy, and B. J. Kennady.** 1980. Cisdiaminedichloroplatinum (DDP) toxicity to the human body kidney reduced by circadian timings. Proc. Am. Soc. Clin. Oncol. **21:**45.
12. **Jehl, F., C. Gallion, and J. Debs.** 1985. High performance liquid chromatographic method for determination of ciprofloxacin in biological fluids. J. Chromatogr. **339:**347–357.
13. **Koopman, M. G., G. M. Koomen, R. T. Kredict, E. A. M. De Moor, F. J. Hock, and L. Arisz.** 1989. Circadian rhythm of glomerular filtration rate in normal individuals. Clin. Sci. **77:**105–111.
14. **Markiewicz, A., and K. Semnowicz.** 1979. Chronokinetics of intestinal absorption and urinary excretion of xylose. Chronobiologia **6:**129.
15. **Mattok, G. L., and J. McGilvery.** 1973. The effect of food intake and sleep on the absorption of acetaminophen. Rev. Can. Biol. **32:**77–84.
16. **Ollagnier, M., H. Deconsus, Y. Cherrah, F. Levi, M. Machkouri, P. Queneau, and A. Reinberg.** 1987. Circadian changes in the pharmacokinetics of oral ketoprofen. Clin. Pharmacokin. **12:**367–378.
17. **Rao, B. R., and D. Rambhau.** 1992. Chronopharmacokinetics of sulphamethoxazole in human volunteers. Drug Invest. **4:**199–204.
18. **Reinberg, A., J. Clinch, J. Ghata, F. Halberg, C. Abulker, J. Dupont, and Z. Zagula Mally.** 1975. Rythmes circadiens des paramétres de l'excrétion urinaire du salicylate (chronopharmacokinétique) chez l'homme adulte sain. C. R. Acad. Sci. (Paris) **280:**1697–1700.
19. **Reinberg, A., and M. H. Smolensky.** 1982. Circadian changes in drug disposition in man. Clin. Pharmacokinet. **7:**401–420.
20. **Rocher, B. A., C. H. Frowirth, A. Gorcein, and A. Reinberg.** 1986. Once a day dosing (morning versus evening) of ethyl loflazepate in anxiety disorders: changes in the circadian rhythm of self rated symptoms. Annu. Rev. Chronopharm **3:**433–436.
21. **Roy, A. B.** 1971. Handbook of experimental pharmacology, vol. 28. Springer-Verlag, Berlin.
22. **William, R. T.** 1967. The biogenesis of conjugation and detoxification products, p. 589–639. *In* P. Bernfeld (ed.), Biogenesis of natural compounds. Pergamon Press, Oxford, N.Y.